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Growth Factors and Extracellular Matrix in Glomerular Disease

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Growth Factors and Extracellular Matrix in Glomerular Disease

Research Plan

A. Specific Aims

Accumulation of extracellular matrix (ECM) in the glomerulus is an important finding in most forms of experimental glomerular injury and many cases of human glomerulonephritis. Preliminary data from our laboratory suggests that transforming growth factor beta (TGFβ) may be responsible for this ECM accumulation. We have shown that when TGFβ is added to cultured rat mesangial cells, it dramatically increases the production of two chondroitin/dermatan sulfate proteoglycans (biglycan and decorin) without altering synthesis of other matrix glycoproteins. Other growth factors tested had no effects. The unique effect of TGFβ on ECM proteoglycan production may be viewed as the specific ECM "footprint" of TGFβ. When this "footprint" is found in studies of ECM it can signify that TGFβ is/has been present. We have analyzed ECM production in a model of mesangial cell injury in the rat and have found the "footprint" of TGFβ. That is, the injured glomeruli are synthesizing increased amounts of biglycan and decorin; and, when conditioned media from the glomeruli are added to cultures of mesangial cells, biglycan and decorin production is strikingly stimulated. The objective of the proposed studies is to test the hypothesis that growth factors (TGFβ) regulates the production and accumulation of ECM in glomerular disease

1. In vitro:
 - a. To determine the effects of TGFβ and other growth factors on the production and cell matrix deposition of proteoglycans and ECM glycoproteins by cultured rat mesangial cells.
 - b. To determine the ability of anti-TGFβ antibodies and other agents to block the action of TGFβ on ECM production.
2. In vivo:
 - a. To further develop an animal model of specific mesangial cell injury and ECM expansion.
 - b. To characterize the composition of the pathologic ECM in tissue.
 - c. To characterize the production of ECM by diseased glomeruli in culture and correlate with growth factor induced changes in cultured mesangial cells.
 - d. To identify the production of growth factors by cultured mesangial cells and glomeruli using conditioned media in biological and radioimmunoassays.
 - e. To identify the presence of growth factors in diseased glomeruli by Northern analysis and in situ hybridization.
 - f. To develop regimens for therapeutic intervention in the disease model by antibodies and other agents capable of neutralizing the TGFβ effect.

B. Background and Significance

The studies we propose are designed to answer two related questions that have general relevance to the pathogenesis of glomerular disease: 1) Is the production of extracellular matrix (ECM) by glomerular mesangial cells influenced by growth factors? 2) Is the observed accumulation or expansion of mesangial matrix in a defined disease model due to the effects of growth factors? We will review background information pertinent to these two questions.

1. Overview of the Relationship of Glomerular Injury to Glomerulosclerosis:
Acutely, glomerular injury in both humans and animals is manifested by varying degrees of hypercellularity, proteinuria, hematuria,

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(93). mRNA will be isolated from diseased glomeruli at days 1, 4, 7, 14 and 28 post anti-thymocyte antibody injection and, along with mRNA from normal kidneys, measured by Northern hybridization. β actin mRNA will be used as a control to exclude nonspecific stimulation of synthesis rates (64). If we find increased expression of TGF β -1 mRNA, we will extend the studies to include the other dimers, TGF β -2 and TGF β -1.2.

We will also use our experience and technique of Northern analysis to study the expression of biglycan, decorin, fibronectin and tenascin. The respective probes will be provided by Dr. Ruoslahti's laboratory. It is possible that the expression levels of fibronectin and tenascin will be insufficiently elevated to be significant; therefore, we anticipate that our emphasis will be on the proteoglycans. We expect to find that anti-thymocyte antibody induces increased expression of TGF β message and that this will be followed by increased expression of biglycan, decorin and, perhaps, tenascin mRNA.

d. Identification of Glomerular Cells Producing TGF β -1. An important step in investigating the model of mesangial injury will be to identify which glomerular cells are producing TGF β mRNA. We will use the cDNA probe (93) to perform *in situ* hybridization on kidney tissue. This approach has been used successfully to demonstrate TGF β message in mouse epidermis (94) and in tissue macrophages (95). The experiments will identify which cells are producing TGF β and provide insight into the importance of infiltrating cells as a source of growth factors.

e. Therapeutic intervention in disease model. We have proposed several experiments that may provide agents that could block or ameliorate the action of TGF β in the animal model of mesangial injury. This includes: 1) verification of the antagonistic effects of PDGF and/or other growth factors, 2) effects of the RGD peptide and 3) the production of neutralizing antisera. It is conceivable that one or more of these agents could be administered to the animal and/or infused directly into the kidney as therapeutic agents to prevent the expansion of mesangial matrix. Our laboratory is experienced in this type of intervention in animal models of glomerular disease (85-88). We expect that one or more of the agents to be tested will block the action of TGF β . This information would be immediately applicable to the design of a study to treat humans with glomerulonephritis.

3. Schedule of Proposed Studies:

- a. Year 1. Conduct cell culture experiments to fully characterize TGF β 's effect on ECM. Determine composition of cell surface ECM following exposure to TGF β . Study mixtures of growth factors to identify synergistic and/or antagonistic effects on ECM production. Characterize composition of mesangial matrix in the animal model and determine ECM components produced by cultured glomeruli and *in vivo* labeled glomeruli. Develop antibody to TGF β and perfect radioimmunoassay.
- b. Year 2. Measure levels of TGF β in diseased glomerular conditioned media. Block TGF β effect on mesangial cell proteoglycan production with antibody. Perfect Northern hybridization and measure TGF β mRNA in kidney tissue. Develop *in situ* hybridization techniques.

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